

CLAIMS

1. Vector material characterised in that said material contains:
- a tumour cell sensitizing gene or genes of which expression in a tumour cell yields a sensitizing gene expression product having a potential to cause tumour cells to be killed and destroyed, or to be eliminated, or otherwise to be inactivated, or to be rendered sensitive and/or vulnerable to destruction;
 - a sensitizing gene expression regulatory system, including promoter means, for said sensitizing gene or genes;
 - at least one control gene; and
 - a control gene expression regulatory system responsive in use in a transfected cell to the effect of a predetermined exogenous or endogenous expression inducing influence so as to induce expression of said control gene to yield an expression product having a capacity to establish an operative linkage between said sensitizing gene expression regulatory system and said sensitizing gene or genes effective to trigger and switch on or permit continuous or permanent expression of the latter to bring about continuous production of said sensitizing gene expression product.
2. Vector material as claimed in Claim 1 wherein the tumour sensitizing gene expression product is an enzyme or other bioactive agent that can convert a predetermined inactive prodrug into an active cytotoxic drug.
3. Vector material as claimed in Claim 2 wherein the tumour-sensitizing expression product is a prodrug activating enzyme selected from the following:
- HSV thymidine kinase, nitroreductase, cytosine deaminase, cytochrome p450 2E1 or cytochrome p450 2DVI.
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4. Vector material as claimed in Claim 1 wherein the tumour-sensitizing expression product is an immune-response modifying agent selected from the expression products of the following:

5 GM-CSF, IFN-alpha, IFN-beta, IFN-gamma, IL-1beta, IL-2, IL-4, IL-6,
IL-8, IL-10, IL-12, IL-15, TNFalpha.

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5. Vector material as claimed in any one of the preceding claims wherein the control gene encodes a recombinase enzyme that acts on recombinase target sites to modify the vector material to establish said operative linkage between the sensitizing gene expression regulatory system and the sensitizing gene or genes.

6. Vector material as claimed in Claim 5 wherein the control gene and the recombinase target sites are part of a Cre-loxP or a Flp-frt site specific recombination system.

7. Vector material as claimed in Claim 5 or 6 wherein said recombinase target sites are separated by a region containing a "stop" sequence of nucleotides that blocks or otherwise prevents expression of the sensitizing gene or genes until removed by the action of said recombinase enzyme.

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8. Vector material as claimed in Claim 5 or 6 wherein the protein coding regions of the sensitizing gene or genes are operationally separated from the said promoters and wherein said recombinase target sites are arranged such that recombination brings about the juxtapositioning of the sensitizing gene promoters and protein coding regions of the sensitizing gene or genes resulting in their expression.

9. Vector material as claimed in any one of Claims 5 to 8 wherein the control gene is a fusion gene that when expressed produces a fusion protein consisting of a recombinase and an intercellular trafficking protein (such as for example the virion protein VP22).

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10. Vector material as claimed in any one of Claims 5 to 9 wherein the region between said recombinase target sites contains a duplicate copy of the recombinase control gene together with an associated promoter.
11. Vector material as claimed in any one of the preceding claims wherein 5 the sensitizing gene expression regulatory system incorporates at least one expression inducible element responsive to the effect of a predetermined exogenous or endogenous expression inducing influence.
12. Vector material as claimed in any one of the preceding claims wherein 10 the sensitizing gene is a fusion gene that when expressed produces a fusion protein consisting of a sensitizing protein and an intercellular trafficking protein.
13. Vector material as claimed in any one of Claims 1 to 8 wherein the or 15 each tumour sensitizing gene is selected from the *E.coli* nitroreductase gene, cytosine deaminase (CD) gene, *Herpes simplex* virus thymidine kinase (HSV-*tk*), mammalian cytochrome p450 2E1 or 2DVI gene, and their functional equivalents.
14. Vector material as claimed in any one of the preceding claims wherein 20 the tumour cell sensitizing gene or genes and the control gene are in separate vectors.
15. Vector material as claimed in any one of Claims 1 to 13 wherein the 25 tumour cell sensitizing gene or genes and the control gene are in the same vector.
16. Vector material as claimed in any one of the preceding claims for use in antitumour therapy characterised in that the use comprises the introduction of 25 the material into tumour cells.
17. Vector material as claimed in Claim 16 wherein said expression inducing influence is endogenous and tumour related, being produced by

tumour cells associated specifically with tumours to which said antitumour therapy is directed.

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18. Vector material as claimed in Claim 17 further characterised in that at least one element of the control gene expression regulatory system is selected so that the control gene is automatically upregulated to an effective operational level when the vector material is introduced into cells of said tumours.
19. Vector material as claimed in Claim 18 wherein the selection of said at least one element of the control gene expression regulatory system is carried out using gene array technology.
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20. Vector material as claimed in any one of Claims 17 to 19 wherein said control gene expression regulatory system responds in use in a transfected cell to said expression inducing influence where the latter is provided by a change in environmental thermal conditions in cells containing the vector material.
21. Vector material as claimed in any one of Claims 17 to 19 wherein said control gene expression regulatory system responds in use in a transfected cell to said expression inducing influence where the latter is provided by a change in local oxygen concentration.
22. Vector material as claimed in any one of Claims 16 to 19 wherein said control gene expression regulatory system includes an expression control element responsive in use in a transfected cell to a hypoxia condition in the cellular environment.
23. Vector material as claimed in Claim 16 wherein said control gene expression regulatory system responds in use in a transfected cell to said expression inducing influence where the latter is provided by an exogenous expression inducing agent applied to cells into which the vector material is introduced.

24. Vector material as claimed in Claim 23 wherein the dose level of said exogenous expression inducing agent needed to trigger and switch on expression of said sensitizing gene or genes in the cells of tissue into which the vector material has been introduced is substantially sub-lethal.
- 5 25. Vector material as claimed in Claim 23 or 24 wherein said control gene expression regulatory system responds in use in a transfected cell to said expression inducing agent which is provided by at least one of the following:
- 10 electromagnetic radiation, application of heat or cooling, application of a magnetic or electric field, an exogenous chemical inducing agent, radiation in the form of sub-atomic particles.
- 15 26. Vector material as claimed in Claim 25 wherein said expression inducing agent is electromagnetic radiation in the form of ultra-violet or visible light.
- 20 27. Vector material as claimed in Claim 25 wherein said expression inducing influence is electromagnetic radiation in the form of X-rays or gamma-rays.
28. Vector material as claimed in Claim 25 wherein said expression inducing agent is electromagnetic radiation in the form of X-rays or gamma-rays at a substantially sub-lethal dosage.
29. Vector material as claimed in Claim 23 or 24 wherein said expression inducing agent is an exogenous chemical expression inducing agent that induces cellular damage.
30. Vector material as claimed in Claim 23 or 24 wherein said expression inducing agent is an exogenous chemical expression inducing agent in the form of an antitumour drug.

31. Vector material as claimed in Claim 30 wherein the antitumour drug is a platinum containing drug.

32. Vector material as claimed in Claim 30 wherein the antitumour drug is selected from the following:

5 Temozolomide, Dacarbazine, Streptozotocin, Procarbazine, Carmustine, Semustine, Lomustine, Fotemustine, Busulphan, Treosulphan, Mechlorethamine, Cyclophosphamide, Iphosphamide, Chlorambucil, Melphalan, ethyleneimines triethylene melamine, hexamethylmelamine, TEPA and thio-TEPA, dibromomannital and dibromodulcitol, hydroxyurea, Methotrexate, azaserine Azathioprin, 5-azacytidine, 5-fluorouracil, cytosine arabinoside, 6-mercaptopurine, Allopurinol 6-thioguanine, deoxycoformycin, Tiazofurin, Acivicin, Pyrazofurin and p-aminolaevulinic acid, plant alkaloids such as Vinblastine, Vincristine and Vindesine, Etoposide and Teniposide, antitumour antibiotics such as Doxorubicin, Daunorubicin, Actinomycin, Bleomycins, Mytomycin, Mythramycin, Mitozantrone hormones such as oestrogen and progesterone.

10 15 20 25 33. Vector material as claimed in Claim 23 or 24 wherein said expression inducing agent is an exogenous chemical inducing agent in the form of a hormone that interacts with a receptor molecule which interacts with a complementary hormone responsive element in the control gene expression regulatory system.

34. Vector material as claimed in Claim 23 or 24 wherein the control gene expression regulatory system comprises a gene upregulation system that can be activated by a chemical agent.

35. Vector material as claimed in Claim 22 or 23 containing number of different control gene expression regulatory elements responsive to different

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expression inducing influences so as to be activated under a range of different conditions.

36. Vector material as claimed in any of the preceding claims wherein at least one element of the sensitizing gene expression regulatory system is 5 inducible in response to the effect of a predetermined exogenous or endogenous expression inducing influence.

37. Vector material as claimed in Claim 36 wherein the sensitizing gene expression regulatory system includes an expression control element responsive in use in a transfected cell to a hypoxic condition in the cellular environment.

10 *Sub A* 38. Vector material as claimed in any of the preceding claims wherein at least one element of the sensitizing gene expression regulatory system is selected for efficiency in the particular tumour(s) to which said antitumour therapy is directed, the selection being carried out using gene array technology.

15 39. Vector material as claimed in any of the preceding claims which includes a plurality of tumour sensitizing genes providing a range of different expression products.

20 40. Vector material as claimed in Claim 39 wherein the different expression products are prodrug activating agents which are effective in relation to a range of different prodrugs, or which provide a range of different immune system stimulatory factors.

Sub A 41. A pharmaceutical composition comprising vector material as claimed in any of the preceding claims in association with a pharmaceutically acceptable carrier or excipient.

25 42. A pharmaceutical composition as claimed in Claim 41 which further includes a transfection agent.

Sub A 43. A kit comprising one or more unit doses of vector material as defined in any one of Claims 1 to 40 together with a transfection agent.

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44. A kit comprising:
- (a) a vector which comprises a tumour cell sensitizing gene or genes and a sensitizing gene expression regulatory system as defined in any one of the Claims 1 to 40;
- 5 (b) a vector which comprises a control gene and a control gene expression regulatory system as defined in any one of Claims 1 to 40; and
- (c) instructions for the use of vectors (a) and (b) in antitumour therapy.
45. A kit comprising:
- 10 (a) vector material as defined in any one of Claims 1 to 40;
- (b) a vector which comprises a tumour cell sensitizing gene or genes and a sensitizing gene expression regulatory system as defined in any one of Claims 1 to 40.
46. A kit as claimed in Claim 44 or 45 for use in antitumour therapy.
- 15 47. A kit as claimed in Claim 43, 44, 45 or 46 wherein each of the vectors and/or vector material is provided in the form of a pharmaceutical composition in association with a pharmaceutically acceptable carrier or excipient.
48. A kit as claimed in any one of Claims 43 to 47 wherein the sensitizing gene(s) produce(s) a prodrug activating agent and wherein one or more doses of 20 a prodrug matched to said prodrug activating agent are also included in said kit.
49. A method of treatment for cancer patients wherein there is delivered to tumour cells vector material as claimed in any of Claims 1 to 40, said cells then being subjected to the appropriate expression inducing influence.
50. A method of treating tumour cells in a biological host system 25 comprising:

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- (a) administering to the system an effective amount of a composition comprising vector material containing a tumour cell sensitizing gene or genes and having a control gene expression regulatory system responsive to a predetermined exogenous or endogenous expression inducing influence, said control gene expression regulatory system being operatively linked to a recombinase gene, together with recombinase target sites flanking a region of which removal permits continuous expression of said tumour cell sensitizing gene(s) as specified above, or primes said sensitizing gene(s) for continuous induced expression;
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- (b) causing said tumour cells transfected with said vector material to be subjected to a dose of an expression inducing agent effective to activate the recombinase gene expression control system of said vector material, thereby to bring about, via recombinase-mediated site specific recombination within the vector material, expression of the or each tumour cell sensitizing gene component;
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- and, in the case of prodrug activating sensitizing genes,
- (c) administering to the host an effective amount of a composition comprising a prodrug convertible into an active form by the expression product of said tumour cell sensitizing gene or genes.
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51. Use of vector material as defined in any one of Claims 1 to 40 in the manufacture of a medicament or of a kit as defined in Claim 43 or 45 for use in antitumour therapy.
52. Use of a vector which comprises a tumour cell sensitizing gene or genes and a sensitizing gene expression regulatory system as defined in any one of Claims 1 to 40 in the manufacture of a kit as defined in Claim 44 for use in antitumour therapy.

53. Use of a vector which comprises a control gene and a control gene expression regulatory system as defined in any one of Claims 1 to 40 in the manufacture of a kit as defined in Claim 44 or 45 for use in antitumour therapy.